Consideration of "Dose" in Evaluation of ToxCast™ Data: Use of Biomonitoring and Pharmacokinetic Data

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Abstract

The purpose of the ToxCast™ program is to evaluate a series of *in vitro* assays for their predictive power against existing toxicological data sets, with the ultimate goal of validating assay(s) for use as screening tools to prioritize chemicals for further toxicological testing and risk assessment. The value of the assays as screening tools will be improved if interpretations of responses can be made not only in terms of hazard, but also in terms of exposure or dose. Translation of *in vitro* concentrations to corresponding relevant external exposure levels requires substantial pharmacokinetic data. However, initial screening of the concentration ranges associated with responses may be made by comparison to a) measures of animal blood or plasma concentrations in previous studies, and/or b) human biomonitoring data. Such evaluations may contribute to the understanding of the sensitivity of the ToxCast™ assays as well as to the relevance of the tested concentration(s). Case studies are presented in which the tested and responding concentrations *in vitro* from the ToxCast™ dataset are compared to measured *in vivo* blood or plasma concentrations from population biomonitoring studies and to Biomonitoring Equivalents (blood or plasma concentrations of chemicals that correspond with existing exposure guidance values such as Reference Doses, etc.). Case study compounds include 2,4-dichlorophenoxyacetic acid and di(2-ethylhexyl) phthalate. We discuss recommendations for further data mining from data collected by the Office of Pesticide Programs, upcoming datasets from the NHANES program, and potential strategies for identifying and/or developing relevant *in vivo* concentration data for use in further screening-level evaluation of datasets from ToxCast™ and other high-throughput screening assays.

Background

Immediate Goals – ToxCast

- Compare pattern of responses in *in vitro* assays included in the ToxCast program to pattern of responses observed *in vivo* for the training set of compounds.
- Based on this validation exercise, develop a set of *in vitro* assays for use in screening new, untested compounds for further, traditional, *in vivo* toxicity testing

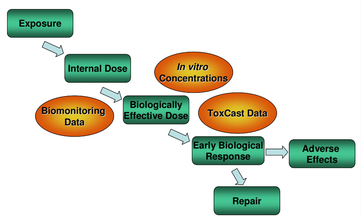
Long-Term Goals – 21st Century Tox Testing

- Reduce or eliminate need for *in vivo* toxicity testing

Consideration of exposure and dose-response is important in both enterprises. Otherwise, evaluations are based only on hazard, and priorities may be misplaced

Placing *in vitro* derived toxicity data into the 'dose-response' continuum requires extrapolations not typically conducted in the current risk assessment model. Extrapolation back to external exposure requires more data than comparison of *in vitro* concentrations to biomonitoring data or measured data on internal dose in experimental animals.

Biomonitoring and pharmacokinetic data may provide additional information to assist in prioritization of compounds for further testing. Integration of such information into the evaluation of the ToxCast datasets requires understanding of factors relating *in vitro* concentrations to relevant *in vivo* exposures.



Methods and Considerations

Biomonitoring data provide measures of internal dose of parent or metabolite that may be relevant to evaluation of the tested *in vitro* concentrations in ToxCast and other *in vitro* systems. Biomonitoring Equivalents (BEs) are estimates of biomarker concentrations that are consistent with existing exposure guidance values such as Reference Doses (RfDs) and their underlying point of departure. BEs may be derived from pharmacokinetic models or from measured concentrations in laboratory animals at or near the point of departure.

BE values and other internal dose data may help inform the evaluation of ToxCast datasets. Specifically, in the validation phase of the ToxCast program, evaluations of responding concentrations *in vitro* in comparison to internal dose benchmarks such as BE values or measured biomonitoring data may help in the evaluation of the sensitivity of the ToxCast assays across compounds. These evaluations can address questions including:

1. Are the tested concentrations physiologically relevant?
2. Are the responding concentrations physiologically relevant, either in the context of the existing whole animal toxicology data or in the context of actual population exposures?
3. Is there a consistent relationship between *in vivo* concentrations of interest and responding concentrations *in vitro*?

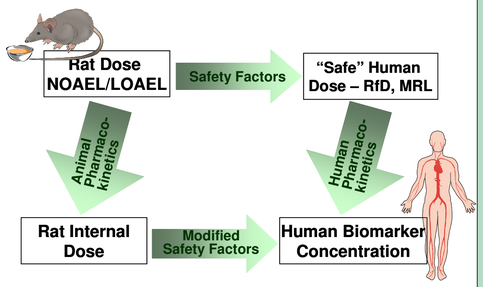


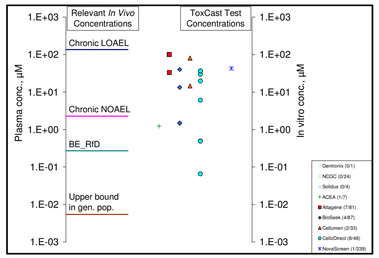
Figure 1: Schematic depicting the concept of Biomonitoring Equivalents- biomarker concentrations consistent with existing risk-assessment-derived exposure guidance values.

Case Studies

Relevant *in vivo* concentrations of interest were identified for three of the ToxCast chemicals: 2,4-Dichlorophenoxyacetic acid (2,4-D); mono(ethylhexyl)phthalate (MEHP, the mono-ester metabolite of di(2-ethylhexyl)phthalate); and perfluorooctanoic acid (PFOA). These concentrations are compared to the responding concentrations in the ToxCast assay dataset. The current effort does not include evaluation of the pattern of responses with respect to effects, but instead focuses first on the physiological relevance of the tested and responding concentrations.

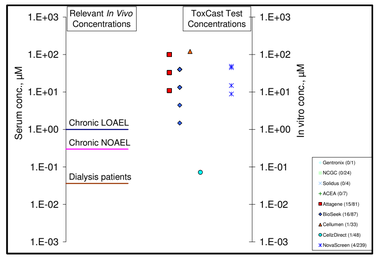
Results

These figures present physiologically relevant serum or plasma concentrations on the left and responding *in vitro* concentrations (lowest effect levels or EC50 values, depending on the assay) on the right. The number of responding endpoints out of tested endpoints are presented in the legend for each ToxCast assay. Responding endpoints are represented by symbols in the right half of the graphs.



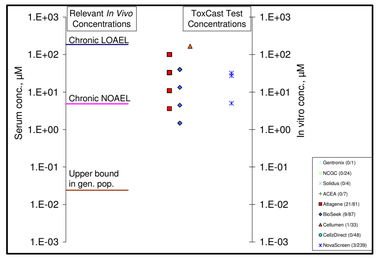
2,4-D

The plasma concentrations associated with the chronic NOAEL and LOAELs in rats were measured by Saghir et al. (2006). The BE_RfD is derived and presented by Aylward et al. (2008). Upper bound concentrations in the general population are estimated based on extrapolation from urinary biomonitoring data in NHANES using available human PK data to estimate corresponding plasma concentrations.



MEHP

The serum concentrations associated with the chronic NOAEL and LOAELs for DEHP in rats (used as the basis for TDI derivation by the European Food Safety Authority) are average concentrations estimated based on Kessler et al. (2004); peak concentrations could be somewhat higher. Data on MEHP serum concentrations in dialysis patients were taken from Mettang et al. (1999).



PFOA

The serum concentrations associated with the chronic NOAEL (in rats) and LOAEL (in monkeys) were reported in the review by USEPA (2005). Upper bound of general population data is based on NHANES biomonitoring data (Calafat et al. 2007)

Observations and Next Steps

Observations from Case Studies

Responding concentrations *in vitro* tended to occur in the range of physiologically relevant concentrations for these three compounds.

- For 2,4-D, one assay (CellzDirect) showed responses substantially below the plasma concentration associated with the chronic NOAEL
- For MEHP, one assay (CellzDirect) showed a single response below the serum concentration associated with the chronic NOAEL. Remaining responding endpoints generally responded only at concentrations substantially higher than the LOAEL.
- For PFOA, responding concentrations were generally in a physiologically relevant range, from approximately the chronic NOAEL to the chronic LOAEL.

Suggested steps during the validation phase of ToxCast:

Evaluation of tested and responding *in vitro* concentrations will be informed through various steps:

1. Mine published data sets to identify estimated internal dose concentrations of interest (blood, tissue) for ToxCast chemicals.
2. Review OPP Data Evaluation Reports (DERs) to identify additional pharmacokinetic data relevant to evaluations
3. Integrate NHANES blood-based biomonitoring data as it becomes available.
4. Consider chemical characteristics and mechanistic information relevant to interpretation. These include:
   - Lipophilicity
   - Protein binding
   - Volatility
   - Cellular transport mechanisms relevant to the chemical
5. Examine cell culture system conditions and media used in the ToxCast assays in comparison to physiological environment:
   - Cell culture medium
     - Lipid content
     - Protein content
     - Other characteristics
   - Carrier or solvent chemicals used in the process that may influence transport into the cells (e.g., DMSO)
   - Availability and activity of metabolism in the cell culture compared to *in vivo*
     - Detoxification vs. activation
     - Phase I and Phase II
   - Availability of elimination pathways
   - Time course of *in vitro* experiment
   - Range of tested concentrations *in vitro*
6. Integrate concentration-response information with qualitative information on pattern of observed responses

Acknowledgements

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